UPON THE PRESENCE AND DISTRIBUTION OF A CHROMATOPHOROTROPIC PRINCIPLE IN THE CENTRAL NERVOUS SYSTEM OF LIMULUS[1]

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Certain definite glandular bodies in arthropods have been shown to produce hormone substances. The more important of these are the crustacean sinus gland, located in the eyestalks of a majority of decapod crustaceans, and the corpora allata and the corpora cardiaca located in the vicinity of the esophagus in insects. These glands appear to be concerned with chromatic adaptations, growth, molt, reproduction, metamorphosis, and certain other functions.

Recently it has been pointed out that certain portions of the nervous system act in an endocrine capacity. This has been demonstrated by the work of Kopeć (1922), Brown (1933), Fraenkel (1935), Hosoi (1934), Brown and Ederstrom (1940), Wigglesworth (1940). Furthermore, histological studies of the nervous system of invertebrates as well as vertebrates have shown certain cells and cell clusters whose cytoplasm is definitely filled with granules or colloid, very strongly suggesting glandular activity (See Scharrer and Scharrer, 1940). This latter paper also describes the presence of such neurosecretory cells in *Limulus*.

With these facts in mind, we attempted to discover and measure an endocrine activity of certain tissues in the arachnid *Limulus*. The only work which had been done previously was that of Snyder-Cooper (1938). She was unable to discover any endocrine activity of the eyes, optic nerves, or central nervous system of *Limulus*, using the chromatophore system of *Palaemonetes vulgaris* as a test object. Since there are many chromatophore types in the crustaceans and recent work has demonstrated that the chromatophores show fundamental differences in their responses to known endocrine materials, it appeared worthwhile to reinvestigate the problem using *Limulus* with a number of chromatophore types other than those of *Palaemonetes* as an index of the presence of an active chromatophoric substance.

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The experiments reported here are restricted to a consideration of the activity of the central nervous system, because it appeared to be the most likely place of origin of an endocrine substance should any occur within the group, especially so since a portion of the nervous system has been shown to be active in both the other two classes of arthropods investigated. In this report a chromatophorotropic activity of the nervous system of *Limulus* will be described and it will be demonstrated conclusively that the active principle found within the nervous system is not uniformly distributed throughout the nervous tissue but shows a definite differential distribution. It may be seen in the paper following upon this one (Scharrer, 1941) that this differential distribution can be correlated with the distribution of neurosecretory cells within the central nervous system of the same species.

Materials and Methods

The experiments were commenced at the Marine Biological Laboratory at Woods Hole, Massachusetts, where freshly-caught *Limulus* and *Uca* were available, and completed at Evanston, Illinois, using *Limulus* and *Uca* which had been shipped from Woods Hole.

For the preparation of extracts of the nervous system, the live *Limulus* was quickly opened up, the nervous system removed and placed in sea water in a shallow container. The lateral nerves were trimmed away, leaving only their short stubs attached to the large nerve ring and the longitudinal chain of abdominal ganglia. In order to determine the effectiveness of various regions of the central nervous system, the system was cut with a scalpel into seven portions: section 1 included the anterior portion of the nerve ring; section 2, the lateral portions; section 3, the posterior portion of the nerve ring; sections 4, 5, 6 and 7 included respectively the first, second, third, and terminal ganglionic masses of the longitudinal cord. The relative positions of these cuts through the nervous system can be seen in Fig. 1. Each of the seven portions of the central nervous system was placed in a separate mortar and permitted to dry briefly; the nerve masses were then triturated thoroughly with pestles, in 2 cc. of sea water. It is appreciated that the total volume of extract obtained for the various nerve sections was somewhat different, due to the different sizes of the nerve masses. However, since the largest portion of the nervous system used in our experiments weighed less than .04 gram (except in one animal), this error was not considered an appreciable one. The extracts were then brought to a boil in order to precipitate out protein materials from the solution. The clear supernatant fluid was then used for assay purposes.

With each experimental series a control solution was prepared, consisting of a piece of muscle or digestive tract wall or gonad of approximately the same size as the largest nerve portion, extracted and treated in the same manner as the experimental solutions.

A sample of each extract, including the control, was injected into three blinded *Uca pugnax,* each *Uca* receiving an injection of approximately .05 cc. The injection was made into the basal segment of the third or fourth thoracic appendage.

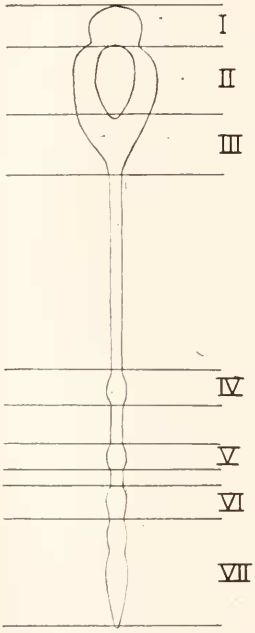

FIG. 1. Diagram of *Limulus* central nervous organs showing the sections of the system which were separately assayed for the chromatophorotropic principle.

Five experimental series were run. The chromatophore index for both black and white chromatophores was recorded at the beginning of each experiment and readings were taken at 15, 30, 45, 60 and 90 minutes. In the first experiment a large *Limulus,* approximately 30 cm. in length from the anterior end of the cephalothorax to the base of the telson, served as the source of nervous tissue. In the remaining four experiments smaller specimens of *Limulus* (about 12 cm. from anterior tip to base of telson) provided the nervous tissue.

Results

The results of these five experiments are shown in tabular form in Tables Ia and Ib. These tables give only the average chromatophore index for the three animals injected with each of the extracts, with the indices for the black and white chromatophores of course averaged separately. In these tables is shown also what has been called the coefficient of effectiveness of the various extracts. This coefficient of effectiveness we realize has only relative significance. It was calculated in the following manner: the sum of the averaged chromatophore indices for each of the two pigments at 15, 30, 45, 60 and 90-minute intervals was obtained. Since an extract having no effect upon the black chromatophores would leave these chromatophores with a chromatophore index of 1 (complete contraction) at each interval—hence a sum of 5—it was considered reasonable to subtract the constant 5 from the sum obtained following injections of active extracts. Similarly, since an extract which would leave the white chromatophores in an initially full dispersed condition (5) would yield a sum of 25, a true index of the effect of an active extract upon the white chromatophores would be the difference between the sum of the average indices and 25.[2] In brief, the coefficient of effectiveness for the black pigment is taken to be $x - 5$ and the coefficient of effectiveness of an extract in concentrating the white pigment is taken to be $25 - x$. In both of these instances x is equal to the sum of the averaged indices. This we believe to be a fair indication of the effectiveness of the extract since it takes into consideration both the magnitude and rate of the response, and, in many cases, duration of the response as well.

Another step was taken to make all the data of the five experiments comparable by obviating the differences which might exist as a result of the different sizes of *Limulus* used for the experiments. This was done by stating the effectiveness of the various portions of the nervous system in terms of the percentage of the effectiveness of Part III (the posterior portion of the ring), which was found in the earliest experiments to be obviously far more effective than any other portion of the nervous system. Thus, in Table I in the column "relative effectiveness" Part III has been arbitrarily assigned an activity of 100 and percentages have been calculated, on the basis of their coefficients of effectiveness, representing the effectiveness of the remaining portions of the nervous system in proportion to Part III.

[2] One difficulty arose which appeared to have no simple solution, namely, that the white chromatophores sometimes initially had their white pigment partially concentrated. It may readily be understood that to the extent to which this is true, the demonstrated differences in concentration of the substance in the nervous system assayed will be minimized.

The results of these experiments are summarized in Table II, in which the relative effectiveness of all the parts of the nervous system and of the control solution have been assembled and averaged. Inspection of these data indicates clearly that Part III is the most active, then,

TABLE Ia

Effect of Extracts upon Uca White

Exp.		0	15	30	45	60	90	Sum	Coefficient of Effectiveness (25−*x*)	Relative Effect
I	I	5.0	2.3	1.5	1.2	1.3	1.5	7.7	17.2	100.0
	II	5.0	2.5	2.5	1.75	1.25	1.0	9.0	16.0	93.0
	III	5.0	2.8	1.5	1.5	1.0	1.0	7.7	17.2	100.0*
	IV	5.0	2.0	3.0	1.8	2.0	2.0	10.7	14.2	82.5
	V	5.0	1.8	2.5	2.5	2.5	2.5	11.7	13.2	76.6
	C	5.0	5.0	5.0	5.0	5.0	5.0	25.0	0.0	
II	I	5.0	1.5	1.4	1.4	1.3	2.2	7.7	17.3	102.2
	II	4.8	2.2	1.9	1.5	1.2	1.1	7.9	17.1	101.2
	III	5.0	2.0	1.7	1.4	1.2	1.8	8.1	16.9	100.0*
	IV	5.0	3.2	3.1	2.8	2.7	3.5	15.3	9.7	57.4
	V	4.5	2.5	2.2	2.0	1.8	2.2	10.7	14.3	84.5
	C	4.0	4.2	4.2	4.1	4.0	4.0	20.5	4.5	26.6
III	I	4.7	2.7	2.7	2.7	2.7	3.0	13.8	11.2	66.2
	II	4.7	2.0	1.8	1.5	1.3	1.7	8.3	16.7	98.7
	III	5.0	2.3	1.8	1.6	1.3	1.1	8.1	16.9	100.0*
	IV	5.0	2.8	2.3	2.5	2.7	2.8	13.1	11.9	70.4
	V	5.0	3.0	3.0	3.4	3.8	4.5	17.7	7.3	43.2
	VI	4.7	3.3	3.3	3.1	3.0	3.4	16.1	8.9	52.6
	VII	3.4	2.0	1.8	1.8	1.8	2.3	9.7	15.3	90.5
	C	4.5	4.5	5.0	4.7	4.5	4.5	23.2	1.8	10.7
IV	I	3.7	1.8	1.8	1.7	1.5	1.5	8.3	16.7	91.3
	II	2.8	1.5	1.5	1.3	1.1	1.1	6.5	18.5	101.0
	III	2.3	1.7	1.5	1.3	1.1	1.1	6.7	18.3	100.0*
	IV	3.5	2.8	2.8	2.7	2.5	2.8	13.6	11.4	62.0
	V	3.8	2.8	2.7	2.7	2.7	3.1	14.0	11.0	60.1
	VI	3.0	2.8	2.7	2.7	2.8	2.8	13.8	11.2	61.1
	VII	3.2	2.7	2.7	2.8	3.0	3.0	14.2	10.8	59.0
	C	2.8	3.7	3.7	3.7	3.7	3.7	18.5	6.5	35.5
V	I	2.8	1.5	1.3	1.4	1.5	1.3	7.0	18.0	102.2
	II	3.2	2.0	1.7	1.4	1.3	1.5	7.9	17.1	97.2
	III	3.2	1.7	1.5	1.4	1.3	1.5	7.4	17.6	100.0*
	IV	2.7	2.7	2.5	2.4	2.3	2.6	12.5	12.5	71.0
	V	3.3	3.2	3.2	3.1	3.0	3.0	15.5	9.5	54.0
	VI	4.3	3.5	3.0	3.0	3.0	3.2	15.7	9.3	52.9
	VII	3.0	2.3	2.2	2.4	2.7	2.7	12.3	12.7	72.3
	C	3.3	3.2	3.3	3.7	4.0	3.5	17.7	7.3	41.5

TABLE Ib

Effect of Extracts upon Uca Black

Exp.		0	15	30	45	60	90	Sum	Coefficient of Effectiveness ($x-5$)	Relative Effect
I	I	1.0	2.5	2.5	3.3	2.7	3.0	14.0	9.0	81.8
	II	1.0	2.0	1.8	2.2	2.7	3.3	12.0	7.0	63.6
	III	1.0	2.3	2.5	2.7	4.3	4.2	16.0	11.0	100.0*
	IV	1.0	2.0	2.5	2.7	2.5	2.8	12.7	7.7	70.0
	V	1.0	3.0	2.5	3.0	3.2	2.3	14.0	9.0	81.8
	C	1.0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0
II	I	1.0	1.7	1.8	1.8	1.8	1.7	8.8	3.8	38.8
	II	1.0	1.5	2.6	3.5	4.5	3.0	15.1	10.1	103.0
	III	1.0	1.8	2.5	3.7	4.4	2.4	14.8	9.8	100.0*
	IV	1.0	1.8	1.6	1.3	1.1	1.0	6.8	1.8	18.3
	V	1.0	1.3	1.3	1.3	1.3	1.0	6.2	1.2	12.2
	C	1.0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0
III	I	1.0	2.2	2.3	2.3	2.2	2.7	11.7	6.7	58.8
	II	1.0	1.5	2.5	3.0	3.3	2.7	13.0	8.0	70.2
	III	1.0	1.5	3.2	3.7	4.2	3.8	16.4	11.4	100.0*
	IV	1.0	1.5	1.7	1.4	1.2	1.0	6.8	1.8	15.8
	V	1.0	1.5	1.3	1.1	1.0	1.0	5.9	0.9	7.9
	VI	1.0	1.3	1.2	1.0	1.0	1.0	5.5	0.5	4.4
	VII	1.0	1.5	1.5	1.3	1.0	1.0	6.3	1.3	11.4
	C	1.0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0
IV	I	1.3	2.3	3.5	3.2	3.0	3.2	15.2	10.2	104.1
	II	1.0	1.3	1.8	2.7	3.7	4.3	13.8	8.8	88.6
	III	1.0	1.8	2.3	3.2	4.2	3.3	14.8	9.8	100.0*
	IV	1.0	1.7	1.7	1.7	1.7	2.0	8.8	3.8	38.8
	V	1.0	1.8	1.8	1.7	1.7	1.1	8.1	3.1	31.6
	VI	1.0	1.1	1.7	1.4	1.1	1.0	6.3	1.3	13.3
	VII	1.0	1.5	1.3	1.2	1.0	1.0	6.0	1.0	10.2
	C	1.0	1.2	1.0	1.0	1.0	1.0	5.2	0.2	2.0
V	I	1.0	2.0	2.8	2.8	2.8	3.2	13.6	8.6	90.5
	II	1.0	2.2	2.8	3.2	3.8	2.6	14.6	9.6	101.0
	III	1.0	1.8	3.0	3.2	3.5	3.0	14.5	9.5	100.0*
	IV	1.0	1.3	1.5	1.3	1.1	1.0	6.2	1.2	12.6
	V	1.0	1.0	1.0	1.0	1.2	1.0	5.2	0.2	2.1
	VI	1.0	1.2	1.2	1.2	1.0	1.0	5.6	0.6	6.3
	VII	1.0	1.5	1.5	1.5	1.5	1.3	7.3	2.3	24.2
	C	1.0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0

in order, Part II and Part I, and finally the nerve tissue of the ganglia of the longitudinal cord. With these data only, it is obviously impossible to determine the concentration of active principle within the various parts of the nervous system, since the portions varied considerably in size.

TABLE II

Relative Effects of Parts of Central Nervous System

Exp.	On White							
	I	II	III	IV	V	VI	VII	Control
I	100.0	93.0	100.0	82.5	76.6	—	—	0.0
II	102.2	101.2	100.0	57.4	84.5	—	—	26.6
III	66.2	98.7	100.0	70.4	43.2	52.6	90.5	10.7
IV	91.3	101.0	100.0	62.0	60.1	61.1	59.0	35.5
V	102.2	97.2	100.0	71.0	54.0	52.9	72.3	41.5
Av.	92.4	98.2	100.0	68.7	63.7	55.5	73.9	22.8
Exp.	On Black							
	I	II	III	IV	V	VI	VII	Control
I	81.8	63.6	100.0	70.0	81.8	—	—	0.0
II	38.8	103.0	100.0	18.3	12.2	—	—	0.0
III	58.8	70.2	100.0	15.8	7.9	4.4	11.4	0.0
IV	104.1	83.6	100.0	38.8	31.6	13.3	10.2	1.0
V	90.5	101.0	100.0	12.6	2.1	6.3	24.2	0.0
Av.	74.8	84.3	100.0	31.1	27.1	8.0	15.3	0.2

To make this calculation, it was necessary to know two more facts: first, the volume of the various parts of the nervous system extracted, and second, the relation between the concentration of active principle within an extract and the calculated coefficients of effectiveness.

In order to answer the first problem, the various parts of the nervous system used were individually weighed prior to their extraction. The results of these weighings are found in Table III.

TABLE III

Weights of Parts

No.	Part	Exp. IV Wgt. Gms.	Exp. V Wgt. Gms.	Average
I	Anterior nerve ring	.0291	.0350	.032
II	Lateral nerve ring	.0310	.0347	.033
III	Posterior nerve ring	.0219	.0306	.026
IV	First ganglion	.0047	.0044	.0046
V	Second ganglion	.0041	.0030	.0036
VI	Third ganglion	.0032	.0061	.0047
VII	Fourth ganglion	.0021*	.0073	.0047
Control	Muscle	.0186	.0246	.022

* Part of ganglion was lost in the preparation of the ganglion for weighing, therefore average should be higher.

An experiment was then designed to determine the relationship between the coefficients of effectiveness and the concentration of the active principle in the extracts. In two of the preceding five experiments, a portion of the extract prepared from Part III was set aside in order to determine the effects of known dilutions upon the two chromatophoric types of *Uca*. In this experiment the extract of Part III was diluted to half its original concentration, then one-fourth, one-eighth, one-sixteenth, one-thirty-second, and one-sixty-fourth. Each dilution stage was injected into three *Uca* just as in the original assay experiments and the coefficient of effectiveness of each dilution was calculated in the same way. The results were expressed as percentages, keeping the original concentration of Part III of the nervous system as 100 per cent with the various dilution stages decreasing according to their coefficients. The results of this experiment with respect to the white chromatophores are seen in Table IVa. and for the black chromatophores in Table IVb.

These data were used for calculating the relative concentration of active principle throughout the nervous system of *Limulus,* as follows: a graph was constructed, the abscissa of which represented the logarithm of the relative concentration and the ordinate the effectiveness in terms of percentage of the original concentration. The results are plotted in Fig. 2. The best smooth curves possible have been drawn through the two series of eight points. With the aid of these plots, it was possible to determine the relative concentration of active chromatophorotropic principle by locating the percentage response on the graph and reading the log of the relative concentration on the abscissa. Using this technique, the relative concentration of active principle for the various portions of the nervous system used in this experiment were calculated.

In Fig. 3 we have plotted together, upon the same abscissa (the segments of the nervous system) but on different ordinates, the weights of the various experimental sections of the nervous system and the apparent relative total quantity of active principle in each part of the nervous system. We have assumed that the specific gravity of all portions of the nervous system is roughly constant, which seems reasonable.

Now the apparent relative quantity of active principle in each part of the nervous system was divided by the weight in grams of that particular portion, and a figure was obtained which indicates the relative concentration of the active principle in these portions. These calculations are summarized in Table V. Inspection of this table indicates that Part III of the nervous system has double the concentration of Part II and nearly four times the concentration of Parts I, IV, V and VII, and nearly ten times the concentration of Part VI.

TABLE IVa

Effect of Dilution on White

	15	30	45	60	90	Sum	Coeff.	Percentage Relative Effect	
Exp. IV									
1	1.7	1.5	1.3	1.1	1.1	6.7	18.3	100.0*	
1/2	2.0	1.7	1.5	1.3	1.5	8.0	17.0	93.0	
1/4	2.0	1.5	1.6	1.7	2.2	9.0	16.0	87.5	
1/8	1.7	1.8	1.8	1.8	1.7	8.8	16.2	88.5	
1/16	2.2	2.5	2.6	2.7	2.3	12.3	12.7	69.4	
1/32	2.3	2.7	2.5	2.3	2.5	12.3	12.7	69.4	
1/64	3.7	3.3	3.7	4.0	4.0	18.7	6.3	34.4	
0	3.7	3.7	3.7	3.7	3.7	18.5	6.5	35.5	
Exp. V									Average
1	1.7	1.5	1.4	1.3	1.5	7.4	17.6	100.0*	100.0
1/2	1.8	1.5	1.4	1.3	1.3	7.3	17.7	100.8	96.9
1/4	2.2	2.3	2.0	1.7	1.5	9.7	15.3	87.0	87.3
1/8	2.2	2.0	1.9	1.8	2.2	10.1	14.9	84.7	86.6
1/16	2.2	2.2	2.0	1.8	1.8	10.0	15.0	85.3	77.4
1/32	3.5	3.2	3.2	3.2	3.2	16.3	8.7	49.5	59.5
1/64	3.0	2.5	2.6	2.6	2.6	13.3	11.7	66.5	50.5
0	3.2	3.3	3.7	4.0	3.5	17.7	7.3	41.5	38.5

TABLE IVb

Effect of Dilution on Black

	15	30	45	60	90	Sum	Coeff.	Percentage Relative Effect	
Exp. IV									
1	1.8	2.3	3.2	4.2	3.3	14.8	9.8	100.0*	
1/2	2.2	2.3	2.7	3.2	3.2	13.6	8.6	87.7	
1/4	2.8	3.8	3.5	3.2	3.0	16.3	11.3	113.0	
1/8	2.3	2.3	2.4	2.5	2.3	11.8	6.8	69.4	
1/16	1.8	1.7	1.6	1.5	1.3	7.9	2.9	29.6	
1/32	1.5	1.3	1.3	1.3	1.2	6.7	1.7	17.3	
1/64	1.2	1.3	1.3	1.3	1.2	6.3	1.3	13.3	
0	1.0	1.2	1.0	1.0	1.0	5.2	0.2	2.0	
Exp. V									Average
1	1.8	3.0	3.3	3.5	3.0	14.3	9.3	100.0*	100.0
1/2	2.0	2.3	2.3	2.3	2.6	11.5	6.5	69.9	78.8
1/4	1.5	1.5	1.6	1.7	1.7	8.0	3.0	32.2	72.6
1/8	1.7	1.7	1.9	2.2	1.7	9.2	4.2	45.1	57.2
1/16	1.5	1.5	1.6	1.8	1.7	8.1	3.1	33.3	31.5
1/32	1.3	1.3	1.5	1.7	1.5	7.3	2.3	24.7	21.0
1/64	1.0	1.0	1.0	1.3	1.0	5.3	0.3	3.2	8.2
0	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0	1.0

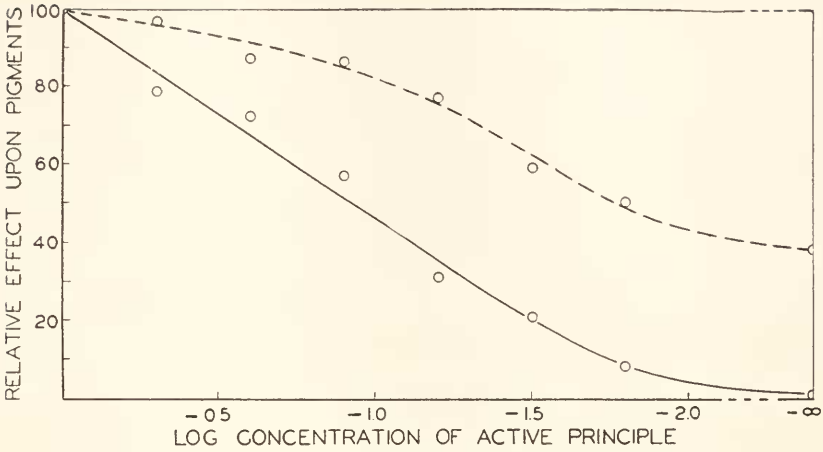

FIG. 2. The relationship between the log concentration of the active principle and the relative effectiveness upon the *Uca* white (dashed line) and black (solid line) pigments.

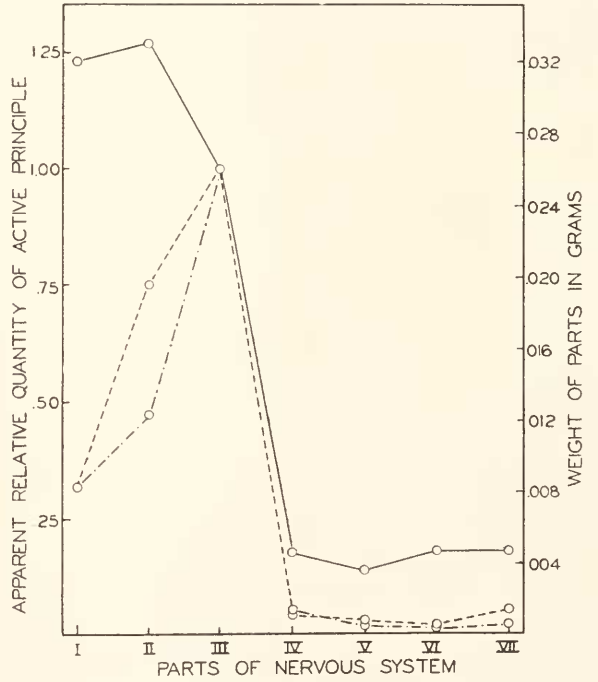

FIG. 3. Plotted together for comparison are the apparent relative quantity of white pigment concentrating principle (dashed line), of black pigment dispersing principle (dot-dash line), and weights of each of the seven assayed parts of the *Limulus* nervous system.

TABLE V

Part	Apparent Rel. Quant. Black Disp. Principle	Apparent Rel. Quant. White Conc. Principle	Weight of Part (gms.)	Rel. Conc. Black Disp. Principle	Rel. Conc. White Conc. Principle	Average
I	0.32	0.32	0.032	10.0	10.0	10.0
II	0.47	0.75	0.033	14.3	22.8	18.6
III	1.00	1.00	0.026	38.5	38.5	38.5
IV	0.053	0.043	0.0045	11.8	9.6	10.7
V	0.045	0.034	0.0035	12.9	9.7	11.3
VI	0.016	0.022	0.0047	3.4	4.7	4.1
VII	0.024	0.054	0.0047	5.1	11.5	8.3

Effects of the Chromatophorotropic Principle of Limulus Nervous System upon Certain Other Chromatophore Types

In the light of the work of Snyder-Cooper (1938) in which she found no apparent chromatophorotropic effects of injection of *Limulus* nervous system extracts upon *Palaemonetes* chromatophores, we believed it worthwhile to repeat her experiments. We were also unable to show any response of either the red or the white chromatophores of *Palaemonetes* to these extracts.

Extracts of the nervous system of *Limulus* were tested upon isolated chromatophores of *Cambarus,* according to the technique of Brown and

TABLE VI

	Uca		*Cambarus*		*Palaemonetes*	
	Black	White	Red	White	Red	White
Sinusgland *Cambarus*	—	—	C	D	—	—
Sinusgland *Uca*	D	D	—	—	C	D
Nervous system *Cambarus*	—	—	C	C	—	—
Corpus cardiacum insects	D	C	C	O	—	—
Brain insect	—	—	O	C	—	—
Nervous system *Limulus*	D	C	O	C	O	O

Meglitsch (1940) and it was found that the chromatophorotropic principle of *Limulus* was very effective in concentrating white pigment, but was entirely without effect upon the red. Furthermore, as Fig. 4 indicates, the relative effectiveness of Parts I through VII was approximately the same upon *Cambarus* white chromatophores as upon *Uca* black and white.

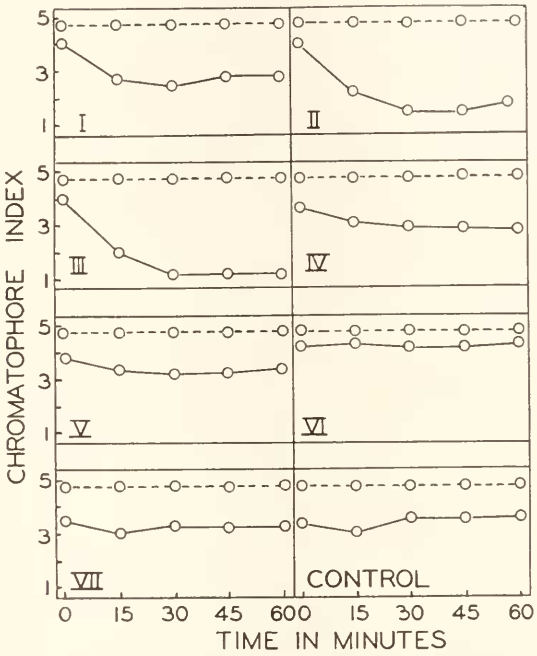

FIG. 4. Relative effects of the parts of *Limulus* nervous system upon crayfish (*Cambarus immunis*) white chromatophores (solid line) and red ones (dashed line). Five upon the ordinate indicates a fully dispersed pigment mass, and one, a fully concentrated pigment mass.

DISCUSSION

Comparison of the Chromatophorotropic Material of Limulus with Chromatophorotropic Materials of Other Arthropods

Table VI has been prepared to show the effects of various arthropod organ extracts upon six types of crustacean chromatophores. This table is admittedly incomplete and although it would be both interesting and instructive to have the gaps filled, it is still possible to draw certain conclusions from it as it is.

In comparing the action of the *Limulus* nerve cord extract with that of the sinus gland extracts of *Uca* and of *Cambarus,* we see that upon *Palaemonetes* red and white chromatophores the *Limulus* extract has no effect, whereas definite and characteristic effects are produced by sinus gland. Upon *Cambarus* red, sinus gland exercises a strong concentrating influence; this is apparently entirely lacking in the *Limulus* extract. Upon *Cambarus* white chromatophores, on the other hand, both the extracts are effective but result in opposite responses of the chromatophore. Similarly, the two extracts have opposite actions upon *Uca* white chromatophores,[3] but upon *Uca* black the activity of the two substances is qualitatively the same.

It seems to us reasonable to assume that this is a similar response of the chromatophore to two chemically different materials, in other words, a non-specific chromatophore reaction. We assume this inasmuch as our experiments suggest that both the white-concentrating action and the black-dispersing action were produced by the same material.

At first it may seem rather extraordinary that *Limulus* should be suspected of having a chromatophorotropic material because of the absence of functional chromatophores in this group of animals, but many other organisms without physiological color change (cockroaches, etc.) possess active corpora cardiaca and sinus glands, and there is abundant evidence accumulated that the chromatophorotropic action of these organs is only one of a number of functions, many of which are far more basic in the life processes of the animals than that of chromatic adaptation. We have utilized the chromatophore response as a test method with a full appreciation of this fact.

There are some who will contend that the materials with which we dealt are nothing more than materials resulting from the mechanical destruction of nerve tissue and possess no normal endocrine function within the organism. This seems highly unlikely considering a number of observations such as the restriction of a specific material to the commissural ganglion of *Crago* and the restriction of a specific action to the corpora cardiaca of insects, and finally, in this research, to a definite demonstration that the material is not uniformly distributed throughout the nervous system, some portions of the nervous system showing roughly ten times the concentration of active principle shown by others. There is some evidence, however, that the material in question in *Limulus* is not produced by a single locus within the nervous system and then

[3] Abramowitz (1937) states that *Uca* eyestalk extract concentrates white pigment in *Uca pugnax*. We have been unable to confirm this observation of Abramowitz and, on the contrary, find that *Uca* eyestalk extract has a definite and striking dispersing action upon white pigment, just as seen in *Palaemonetes* and *Cambarus*.

distributed out from this center by diffusion because it was demonstrated in a brief experiment that the longitudinal commissure connecting the posterior end of the nerve ring with the first ganglion of the longitudinal chain showed significantly lower concentration of active principle (practically no effect) than either the posterior portion of the nerve ring or the ganglion at its opposite end. In the case of diffusion, a smoothly gradual decline in activity would be expected. The increased activity at the posterior tip of the central nervous system also argues against the diffusion of the material out from a single center. Therefore we are inclined to believe that this differential distribution of activity in the nervous system is the result of a differential distribution of cells actively engaged in the production of the substance.

The Probable Number of Hormonal Substances in Limulus Nervous System

If one examines Fig. 3 one is impressed with the parallel nature of quantitative distribution of the black and white pigment-concentrating principles. The differences which occur are not only readily within the experimental error but also are even astonishingly close to one another. On the basis of these data there is no justification for any assumption other than that these two pigments are being affected by one and the same substance. An examination of Fig. 4, showing the effects of the various parts of the nervous system of *Limulus* upon the white chromatophores of *Cambarus*, shows a quantitative gradation of activity of the various parts quite parallel to those shown in Fig. 3 for the *Uca* chromatophores. Again there is apparently no reason for assuming anything other than that the substance active upon *Cambarus* white chromatophores is the same substance responsible for influence on *Uca* chromatophores. We realize the danger of drawing any conclusions upon negative evidence and hence conclude only that there is no suggestion for more than one chromatophorotropic principle in *Limulus* nervous system. However, just as Snyder-Cooper failed to show the presence of any chromatophorotropic principle in *Limulus* using *Palaemonetes* red and white chromatophores, so is it quite possible that utilizing other chromatophores than we have tried will demonstrate the presence of other hormones than we have been able to demonstate.

The action of *Limulus* extract is qualitatively unlike that of extracts of the commissural ganglia and other nervous organs in *Cambarus*, as shown by their effect upon *Cambarus* red chromatophores. The *Cambarus* extracts show an extremely potent activity in concentrating the red pigment while *Limulus* extracts show no effect. On the other hand, the

effects of these two extracts are identical upon *Cambarus* white chromatophores. Two alternative explanations are possible: (1) that the white-concentrating principle from these two sources is similar and that *Cambarus* nervous system possesses in addition a red pigment-concentrating principle, or (2) one could assume that each extract possesses a single principle which is structurally different in the two cases. Again, *Limulus* nerve organ extract differs from extracts of the corpora cardiaca of insects in having a different action upon both red and white chromatophores of *Cambarus*. Finally, in comparing the activity of insect brain and the activity of *Limulus* nervous system, one finds a qualitatively similar action of these two extracts upon both red and white chromatophores of *Cambarus*. Neither possesses an effect on the *Cambarus* red pigment and both exercise a white pigment-concentrating action. Of course it is too soon even to suspect that these latter two substances are identical and further conclusions cannot be drawn until more properties of these two substances have been shown to be identical.

A consideration of these results brings us to a complete realization that a unitary theory of hormonal control of chromatophores in crustaceans—and even more in arthropods in general—is completely untenable. There are undoubtedly several different chromatophorotropic materials found within the various groups, but it is not beyond the realm of possibility that certain threads of similarity or continuity can be woven through various active tissues and their secreted principles in this phylum of animals.

It can be calculated readily that the posterior portion of circumesophageal nerve ring of *Limulus* is still effective when diluted in nearly 5000 times its volume of salt solution. The active secreting cells undoubtedly occupy a very small percentage of the volume of the nervous tissue and consequently, in terms of the ratio of neuro-glandular tissue to volume of extract, the maximal dilution value would be in the hundreds of thousands or even millions.

Summary

1. A principle influencing pigment concentration in *Uca* chromatophores is found in extracts of the central nervous system of *Limulus polyphemus*. This principle is not uniformly distributed through the central nervous system of *Limulus* but is concentrated in the ganglionic masses, with the greatest quantity in the posterior portion of the circumesophageal nerve ring. The lateral portions of the nerve ring show approximately one-half the concentration of the posterior portion and

all the remaining portions of the nervous system show from one-quarter to one-tenth the concentrations of the posterior portion of the nerve ring.

2. The distribution of the principle influencing *Uca* white pigment appears to be identical with that producing dispersion of the *Uca* black pigment and concentration of *Cambarus* white pigment. Hence it is concluded that all three of these effects are brought about by one and the same principle.

3. Certain physiological properties of the chromatophorotropic material from the nervous system of *Limulus* were compared with corresponding properties of certain other invertebrate hormones and it was found that the *Limulus* chromatophorotropic principle is physiologically unlike any other known arthropod hormone substance with the possible exception of insect brain extract.

4. It is calculated that an extract of the posterior portion of the circumesophageal nerve ring is still effective when diluted in nearly 5000 times its volume of salt solution.

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